TOBACCO INDUSTRY RESEARCH COMMITTEE
350 FIFTH AVENUE NEW YORK 1, N. Y.

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Budget Yes

Salaries
Expendable Supplies
Application for Research Grant
Overhead C$
Other Travel

No

Date: April 29, 1955

1. Name of Investigator: Sydney C. Rittenberg

2. Title: Professor of Bacteriology

3. Institution & Address: University of Southern California, Los Angeles 7, California

4. Project or Subject:

Studies on the mechanism of bacterial metabolism of nicotine and related compounds. The ultimate goal of the project is the elucidation of the intermediary metabolism of nicotine oxidation.

5. Detailed Plan of Procedure (Use reverse side if additional space is needed) or sources of supply):

The method of approach would consist of at least a four-phase attack employing, initially, procedures commonly used in metabolic studies.

The first phase would involve a study of the various environmental factors which influence nicotine oxidation by the particular bacterium used (under growing conditions), including the relation to the degree of aerobiosis, the pH, temperature, trace metal balances, and growth factor requirements. The organism would be subjected to variations of one factor, while keeping the others constant and both the extent of growth and the degree of nicotine utilization would be measured. Growth would be determined turbidimetrically, and the nicotine content would be determined chemically by available procedures.

The second phase will consist of an attempt to uncover clues to the metabolic pathway by use of the technique of simultaneous adaptation (Stanier, 1947). Resting cells of the organism (adapted and unadapted to nicotine) will be presented with suspected intermediates, and the adaptive patterns which result will be correlated with a possible metabolic pathway. These studies would be extended to dried cells in order to eliminate permeability as a limiting factor.

Signature /s/ S. C. Rittenberg
Director of Project

The third phase would involve the isolation of intermediates. A two-fold approach will be employed: various metabolic inhibitors will be used, ranging from substances known to inactivate enzyme systems to anti-metabolites, which by virtue of their ability to compete with the substrate for the active sites on the enzyme, would cause an accumulation of intermediates; the second method of attacking this problem would be to subject cells to various conditions

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Additional Information (Cont'd.)

Preliminary studies in our laboratory, using a soil bacterium capable of utilizing nicotine as the sole source of carbon and nitrogen, have indicated that the pyridine ring is oxidized during the oxidation of nicotine. The evidence is based upon the amount of oxygen uptake observed during nicotine oxidation; it has consistently been in excess of that required for the oxidation of the pyrollidine portion of nicotine. The organism employed appears to be similar to those previously reported in the literature (Bucherer. 1942, 1943; Chaman et al. 1954; Gigure. 1954 Wada and Yamaoka.1954) in a number of respects. A blue pigment is produced during oxidation; the oxidation is extremely rapid; morphologically, the organism, a yellow chromogen, appears to be a gram negative rod containing gram positive granules. Using simultaneous adaptation as an analytical tool, preliminary observations have indicated that nicotinic acid and pyridine, as such, are not metabolic intermediates. Resting cells were unable to utilize either of these compounds; dried cells also showed the same pattern.

Basic knowledge elucidating the pathway (or pathways) of nicotine oxidation would be valuable from several viewpoints. First, it would clarify the mechanism whereby a complex ring structure is handled. Secondly, it would enable one to determine how the utilization of the pyrollidine and pyridine rings compares with the utilization of other cyclic compounds. Thirdly, since the quality of tobacco leaves is, in part, determined by their nicotine content, and the latter is greatly influended by the microbial fermentations which occur during curing, it would be of interest to understand the chemistry of the micro-biological processes which occur during curing. Finally, although it does not necessarily follow that the mode of nicotine utilization by bacteria is similar to that in mammalian systems, past experience has indicated that in general, there does exist a great similarity in metabolic pathways among living forms. We see no reason for excluding nicotine from this general picture of Comparative Biochemistry.

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The isolation of these intermediates would be accomplished by the use of chromatographic techniques as well as the chemical isolation of intermediates.

The fourth phase will be on the cell-free level. Cells will be disrupted by various techniques (grinding, ultra-sonic treatment, freeze-thawing, and autolysis) in order to obtain nicotine oxidation by cell-free systems. If these attempts are successful, then fractionation of the enzyme system into its component parts becomes possible with the final characterization and confirmation of each step involved in the oxidation of nicotine.

The methodology, previously outlined, has proven to be successful in the characterization of other oxidative pathways, and no difficulties are anticipated except perhaps in the procurement of synthesis of possible intermediates.

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6. Budget Plan:

Salaries	$2,000
Expendable Supplies	1,500
Permanent Equipment	--
Overhead 8%	304
Other Travel	300
Total	$4,104/year

Application for Grant No

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7. Anticipated Duration of Work: Two years

Henry C. Rittenberg

8. Facilities and Staff Available: ~~Essential~~ AVAILABLE

Essentially all the equipment required for metabolic investigation in our laboratory. No "staff" beyond the project ~~director~~ director and the technical assistance of a graduate student is required. The salary item in the budget is for a graduate student for 2/3 time.

9. Additional Requirements: None

10. Additional Information (Including relation of work to other projects and other sources of supply):

Although it is known that approximately 90% of the ~~nicotine~~ nicotine absorbed by man is metabolized (Larson, 1952), the exact mechanism by which this alkaloid is oxidized is unknown.

Wada and Yamaski (1954) have presented evidence which would indicate that the initial step in the oxidation of nicotine involves ring cleavage between the nitrogen and the 5 position of the pyrollidine ring. They based their conclusions upon the isolation of various intermediates from the growth medium of a soil organism in which nicotine served as the sole source of carbon and energy.

Larson (1952), on the other hand, has postulated that the oxidation of nicotine involves a split of the pyrollidine ring between the nitrogen and the 2 position. His evidence was based upon the color ~~reaction~~ reaction produced by various potential intermediates as compared to a similar color reaction observed in the urine of animals which were administered nicotine. Additional evidence was obtained that the pyridine nucleus was left intact.

Signature /S/ S.C. Rittenberg
Director of Project

/S/ Robert D. Fisher, Financial V.P.
Business Officer of the Institution

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